

**Amendments to the specification:**

Please replace the paragraph beginning at page 34, line 1, with the following rewritten paragraph:

**DNA Extraction and MSP.** Paraffin-embedded primary tumor specimen blocks were sectioned at 10  $\mu$ m deparaffinized in 100% xylene, followed by 100% ethanol incubation and stained with hematoxylin and eosin (H&E). Tumor tissue was microdissected in comparison to a similarly stained and cover-slipped reference slide cut in sequence from each tissue block. The samples were incubated in buffer containing SDS-proteinase K for 48 hr at 50°C with an additional 1  $\mu$ g proteinase K added twice within each 24 hr period. DNA was extracted and bisulfite modification was performed using the agarose bead technique as previously described [Spugnardi, 2003 #86]. Briefly, following extraction, DNA was quantitated using Picogreen (Molecular Probes, Eugene, OR) and 1  $\mu$ g of genomic DNA was mixed with, 0.3 M NaOH, 2 vols of 2% LMP agarose dissolved in molecular grade water, heated at 80°C for 10 min and then added to 2-3 drops of chilled mineral oil to create an agarose bead. Sodium bisulfite conversion of DNA suspended in the agarose bead was achieved by adding 2.5 M sodium metabisulfite and 125 mM hydroquinone and incubating at 50°C for 14 hr. Subsequently, desulphonation was performed by evacuating residual mineral oil and adding 0.2 M NaOH x 2 for 15 min each, followed by neutralization with 1/5 vol 1 M HCL for 5 min and then the bead was washed in Tris-EDTA buffer and stored in molecular grade water at 4°C until analysis. A panel of six genes was assessed for their methylation status: RASSF1A, APC, *Twist*, CDH1, GSTP1 and RAR- $\beta$ 2. MSP was performed on each bead in a 100  $\mu$ l reaction containing 200  $\mu$ M each of dNTP and AmpliTaq Gold DNA polymerase (Perkin Elmer, Norwalk, CT) and 50 pmol of each forward (F) and reverse (R) primer set for methylated (M) and unmethylated (U)

sets as follows: RAR-82, (M) F-GAACGCGAGCGATTTCGAGT (SEQ ID NO:1) and R-GACCAATCCAACCGAAACG (SEQ ID NO:2), (U) F-GGATTGGGATGTTGAGAATGT (SEQ ID NO:3) and R-CAACCAATCCAACCAAAACAA (SEQ ID NO:4); CDH1, (M) F-TTAGGTTAGAGGGTTATCGCGT (SEQ ID NO:5) and R-TAACTAAAAATTACCTACCGAC (SEQ ID NO:6), (U) F-TAATTTTAGGTTAGAGGGTTATTGT (SEQ ID NO:7) and R-CACAACCAATCAACAACACA (SEQ ID NO:8); APC, (M) F-TATTGCGGAGTGCGGGTC (SEQ ID NO:9) and R-TCGACGAACTCCCGACGA (SEQ ID NO:10), (U) F-GTGTTTTATTGTGGAGTGTGGGTT (SEQ ID NO:11) and R-CCAATCACAACTCCCAACAA (SEQ ID NO:12); RASSF1A, (M) F-GTGTTAACGCGTTGCGTATC (SEQ ID NO:13) and R-AACCCCGCGAACTAAAAACGA (SEQ ID NO:14), (U) F-TTTGGTTGGAGTGTGTTAATGTG (SEQ ID NO:15) and R-CAAACCCACAACTAAAAACAA (SEQ ID NO:16); GSTP1, (M) F-TTCGGGGTGTAGCGGTCGTC (SEQ ID NO:17) and R-GCCCAATACTAAATCACGACG (SEQ ID NO:18), (U) F-GATGTTTGGGGTGTAGTGGTTGTT (SEQ ID NO:19) and R-CCACCCCAATACTAAATCACAACA (SEQ ID NO:20); *Twist*, (M) F-TTTCGGATGGGGTTGTTATCG (SEQ ID NO:21) and R-GACGAACGCGAAACGATTTC (SEQ ID NO:22), (U) F-TTGGATGGGGTTGTTATTGT (SEQ ID NO:23) and R-ACCTTCCTCCAACAAACACA (SEQ ID NO:24). PCR was carried out after optimizing annealing temperatures for each primer set to include 40 timed cycles of denaturation at 94°C for 30 sec, annealing for 30 sec, and extension at 72°C for 30 sec. Post-MSP product analysis was performed using capillary array

electrophoresis (CEQ 8000XL Genetic Analysis System, Beckman Coulter, Fullerton, CA) as described previously [Spugnardi, 2003 #86].

Please replace the two paragraphs beginning at page 45, line 12, with the following rewritten paragraphs:

**Genomic Sequencing.** DNA sequences were amplified by mixing 100 ng of bisulfite treated melanoma cell line DNA with 100 pmoles of each respective primer: MGMT, M1 5'-GGGTTATTTGGTAAATTAAGGTATAGAG-3' (SEQ ID NO:25) and M2 5'-CACCTAAAAATAAAACAAAACTACCAC-3' (SEQ ID NO:26); RASSF1A, R3 5'-GGGAGTTTGAGTTTATTGAGTTG-3' (SEQ ID NO:27) and R2 5'-CACCTCTACTCATCTATAACCCAAATAC-3' (SEQ ID NO:28); RAR- $\beta$ 2, RA3 5'-GTGTGATAGAAGTAGTAGGAAGTGAGTTGT-3' (SEQ ID NO:29) and RA2 5'-ACTCCATCAAACCTCTACCCCTTTTTTAAC-3' (SEQ ID NO:30) in a 50  $\mu$ l reaction containing buffer, of dNTP and *AmpliTaq* gold polymerase (Applied Biosystems, Foster City, CA) at 95°C for 45 s, 55°C for 45 s and 72°C for 2 min for 40 cycles. PCR products were gel purified using the QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) and sequenced using an automated DNA sequencer (CEQ 8000XL DNA Analysis System, Beckman Coulter, Fullerton, CA) with the respective internal primer: MGMT, M3 5'-GTTGT(c/t)GGAGGATTAGGGT-3' (SEQ ID NO:31); RASSF1A, R4 5'-TACCCCTTAACCTACCCCTTCC-3' (SEQ ID NO:32), and RAR- $\beta$ 2, RA4 5'-AATCATAAATTATAACAAACAAACCAACT-3' (SEQ ID NO:33).

**Fluorescent MSP Analysis.** Methylation status was assessed for each gene using two sets of fluorescent labeled primers specifically designed to amplify methylated or unmethylated DNA sequence. Primer sequences are listed as methylated sense and antisense followed by unmethylated sense and antisense, with annealing temperatures and PCR product size: TIMP-3, 5'-

CGTTTCGTTATTTTTTGTTCGGTTTC-3' (SEQ ID NO:34) and 5'-  
CCGAAAACCCCGCCTCG-3' (SEQ ID NO:35) (59°C, 116 bp) 5'-  
TTTTGTTTTGTTATTTTTTGTTCGGTTTC-3' (SEQ ID NO:36) and 5'-  
CCCCCAAAAACCCACCTCA-3' (SEQ ID NO:37) (59°C, 122 bp) (19); RASSF1A, 5'-  
GTGTTAACGCGTTGCGTATC-3' (SEQ ID NO:13) and 5'-  
AACCCCGCGAACTAAAAACGA-3' (SEQ ID NO:14) (60°C, 93 bp), 5'-  
TTTGGTTGGAGTGTGTTAATG TG-3' (SEQ ID NO:15) and 5'-  
CAAACCCACAACTAAAAACAA-3' (SEQ ID NO:16) (60°C, 105 bp) (11,16); RAR-  
 $\beta$ 2, 5'-GAACGCGAGCGATTCGAGT-3' (SEQ ID NO:1) and 5'-  
GACCAATCCAACCGAAACG-3' (SEQ ID NO:2) (59°C, 142 bp), 5'-  
GGATTGGGATGTTGAGAATGT-3' (SEQ ID NO:3) and 5'-  
CAACCAATCCAACCAAAACAA-3' (SEQ ID NO:4) (59°C, 158 bp) (Evron et al.,  
2001); MGMT, 5'-TTTCGACGTTTCGTAGGTTTTTCGC-3' (SEQ ID NO:38) and 5'-  
GCACTCTTCCGAAAACGAAACG-3' (SEQ ID NO:39) (66°C, 81 bp), 5'-  
TTTGTGTTTTGATGTTTGTAGGTTTTTGT-3' (SEQ ID NO:40) and 5'-  
AACTCCCACTCTTCCAAAAACAAAAC (SEQ ID NO:41) (66°C, 93 bp) (Esteller et  
al., 1999); DAPK 5'-GGATAGTCGGATCGAGTTAACGTC (SEQ ID NO:42) and 5'-  
CCCTCCCAAACGCCGA (SEQ ID NO:43) (64°C, 98 bp), 5'-GGAGGATA  
GTTGGATTGAGTTAATGTT-3' (SEQ ID NO:44) and 5'-  
CAAATCCCTCCCAAACACCAA-3' (SEQ ID NO:45) (64°C, 106 bp) (Goessl et al.,  
2000); GSTP1, 5'-TTCGGGGTGTAGCGGTCGTC-3' (SEQ ID NO:17) and 5'-  
GCCCCAATACTAAATCACGACG-3' (SEQ ID NO:18) (59°C, 91 bp), 5'-  
GATGTTTGGGGTGTAGTGGTTGTT-3' (SEQ ID NO:19) and 5'-  
CCACCCCAATACTAAATCACAACA-3' (SEQ ID NO:20) (59°C, 97 bp) (Esteller et  
al., 1999; Zochbauer-Muller et al., 2001) ; p16<sup>INK4a</sup>, 5'-  
TTATTAGAGGGTGGGGCGGATCGC-3' (SEQ ID NO:46) and 5'-

GACCCGAACCGCGACCGTAA-3' (SEQ ID NO:47) (65°C, 150 bp), 5'-TTATTAGAGGGTGGGGTGGATTGT-3' (SEQ ID NO:48) and 5'-CAACCCCAAACCACAACCATAA-3' (SEQ ID NO:49) (65°C, 151 bp) (21,24); and MYOD1, 5'-CCAACTCCAAATCCCCTCTCTAT-3' (SEQ ID NO:50) and 5'-TGATTAATTTAGATTGGGTTTAGAGAAGGA-3' (SEQ ID NO:51) (60°C, 162 bp) (Eads et al., 1999). One hundred ng of bisulfite-modified DNA was subjected to PCR amplification in a final reaction volume of 20 µl containing PCR buffer, 2.5-4.5 mM MgCl<sub>2</sub>, dNTPs, 0.3 µM primers, BSA and 0.5 U of *AmpliTaq* gold polymerase (Applied Biosystems). PCR was performed with an initial 10 min incubation at 95°C, followed by 40 cycles of denaturation at 95°C for 30 s, annealing for 30 s, and extension at 72°C for 30 s, and a final seven min hold at 72°C. Sodium-bisulfite modified lymphocytes from healthy donors were used as positive unmethylated control, *SssI* Methylase (New England BioLabs, Beverly, MA) treated modified lymphocytes were used as a positive methylated control, and unmodified lymphocytes were used as a negative control for methylated and unmethylated reactions. PCR products were visualized using capillary array electrophoresis (CAE; CEQ 8000XL). The assay was set up in a 96-well microplate format. Multiple PCR products can be assayed in the same well for comparison. Methylated and unmethylated PCR products from each sample were assessed simultaneously by labeling forward primers with a choice of three Beckman Coulter WellRED Phosphoramidite (PA)-linked dyes (Genset Oligos, Boulder, CO). Forward methylated specific primer was labeled with D4pa dye and forward unmethylated specific primer was labeled with D2pa dye. One µl of methylated PCR product and one µl of unmethylated PCR product were mixed with 40 µl loading buffer and 0.5 µl dye-labeled size standard (Beckman Coulter Inc). The CAE analysis detects the different dyes and displays them in respective colors.

Please replace the paragraph beginning at page 49, line 1, with the following rewritten paragraph:

Realtime PCR assay was performed to obtain the approximate number of methylated gene copies present in a sample. The internal reference gene MYOD1 was used to amplify sodium bisulfite treated DNA independent of methylation status to confirm presence of modified DNA (9). In addition, a standard curve was constructed with serial dilutions of  $10^1$  to  $10^5$  copies of the targeted TSG promoter region template. Copy numbers for the individual samples were established using the standard curve. Primer sequences for the realtime PCR were the same as for the CAE analysis while the probe sequences were as follows: MYOD, 5'-CCCTTCCTATTCCTAAATCCAACCTA-3' (SEQ ID NO:52); MGMT, 5'-CGTTTGCGATTTGGTGAGTGTTTGGG-3' (SEQ ID NO:53); RASSF1A, 5'-CAACTACCGTATAAAATTACACGCGATACCCCG-3' (SEQ ID NO:54); and RAR-β2, 5'-CCGAATACGTTCCGAATCCTACCCCG-3' (SEQ ID NO:55).

Please delete the forty-one paragraphs beginning at page 57, line 19.

Please add the following paragraphs at page 78, line 3:

Baylin, S.B. & Herman, J.G. (2000). *Trends Genet*, 16, 168-174.

Bostick, P.J., Morton, D.L., Turner, R.R., Huynh, K.T., Wang, H.J., Elashoff, R., Essner, R. & Hoon, D.S. (1999). *J Clin Oncol*, 17, 3238-3244.

Chen, C.M., Chen, H.L., Hsiau, T.H., Hsiau, A.H., Shi, H., Brock, G.J., Wei, S.H., Caldwell, C.W., Yan, P.S. & Huang, T.H. (2003). *Am J Pathol*, 163, 37-45.

Christmann, M., Pick, M., Lage, H., Schadendorf, D. & Kaina, B. (2001). *Int J Cancer*, 92, 123-129.

Dammann, R., Li, C., Yoon, J.H., Chin, P.L., Bates, S. & Pfeifer, G.P. (2000). *Nat Genet*, 25, 315-319.

Dammann, R., Yang, G. & Pfeifer, G.P. (2001). *Cancer Res*, 61, 3105-3109.

Davies, H., Bignell, G.R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M.J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., Menzies, A., Mould, C., Parker, A., Stevens, C., Watt, S., Hooper, S., Wilson, R., Jayatilake, H., Gusterson, B.A., Cooper, C., Shipley, J., Hargrave, D., Pritchard-Jones, K., Maitland, N., Chenevix-Trench, G., Riggins, G.J., Bigner, D.D., Palmieri, G., Cossu, A., Flanagan, A., Nicholson, A., Ho, J.W., Leung, S.Y., Yuen, S.T., Weber, B.L., Seigler, H.F., Darrow, T.L., Paterson, H., Marais, R., Marshall, C.J., Wooster, R., Stratton, M.R. & Futreal, P.A. (2002). *Nature*, 417, 949-954.

Demary, K., Wong, L., Liou, J.S., Faller, D.V. & Spanjaard, R.A. (2001). *Endocrinology*, 142, 2600-2605.

Eads, C.A., Danenberg, K.D., Kawakami, K., Saltz, L.B., Danenberg, P.V. & Laird, P.W. (1999). *Cancer Res*, 59, 2302-2306.

Esteller, M., Corn, P.G., Baylin, S.B. & Herman, J.G. (2001). *Cancer Res*, 61, 3225-3229.

Esteller, M., Hamilton, S.R., Burger, P.C., Baylin, S.B. & Herman, J.G. (1999). *Cancer Res*, 59, 793-797.

Esteller, M. & Herman, J.G. (2002). *J Pathol*, 196, 1-7.

Evron, E., Dooley, W.C., Umbricht, C.B., Rosenthal, D., Sacchi, N., Gabrielson, E., Soito, A.B., Hung, D.T., Ljung, B., Davidson, N.E. & Sukumar, S. (2001). *Lancet*, 357, 1335-1336.

Fujiwara, Y., Chi, D.D., Wang, H., Keleman, P., Morton, D.L., Turner, R. & Hoon, D.S. (1999). *Cancer Res*, 59, 1567-1571.

Goessl, C., Krause, H., Muller, M., Heicappell, R., Schrader, M., Sachsinger, J. & Miller, K. (2000). *Cancer Res*, 60, 5941-5945.

Harden, S.V., Tokumaru, Y., Westra, W.H., Goodman, S., Ahrendt, S.A., Yang, S.C. & Sidransky, D. (2003). *Clin Cancer Res*, 9, 1370-1375.

Jeronimo, C., Usadel, H., Henrique, R., Oliveira, J., Lopes, C., Nelson, W.G. & Sidransky, D. (2001). *J Natl Cancer Inst*, 93, 1747-1752.

Johnson, P.J. & Lo, Y.M. (2002). *Clin Chem*, 48, 1186-1193.

Jones, P.A. & Baylin, S.B. (2002). *Nat Rev Genet*, 3, 415-428.

Kurie, J.M., Lotan, R., Lee, J.J., Lee, J.S., Morice, R.C., Liu, D.D., Xu, X.C., Khuri, F.R., Ro, J.Y., Hittelman, W.N., Walsh, G.L., Roth, J.A., Minna, J.D. & Hong, W.K. (2003). *J Natl Cancer Inst*, 95, 206-214.

Lo, K.W., Kwong, J., Hui, A.B., Chan, S.Y., To, K.F., Chan, A.S., Chow, L.S., Teo, P.M., Johnson, P.J. & Huang, D.P. (2001). *Cancer Res*, 61, 3877-3881.

Mangelsdorf, D.J. & Evans, R.M. (1995). *Cell*, 83, 841-850.

Maruyama, R., Toyooka, S., Toyooka, K.O., Harada, K., Virmani, A.K., Zochbauer-Muller, S., Farinas, A.J., Vakar-Lopez, F., Minna, J.D., Sagalowsky, A., Czerniak, B. & Gazdar, A.F. (2001). *Cancer Res*, 61, 8659-8663.

Paz, M.F., Fraga, M.F., Avila, S., Guo, M., Pollan, M., Herman, J.G. & Esteller, M. (2003). *Cancer Res*, 63, 1114-1121.

Pfeifer, G.P., Yoon, J.H., Liu, L., Tommasi, S., Wilczynski, S.P. & Dammann, R. (2002). *Biol Chem*, 383, 907-914.

Rosas, S.L., Koch, W., da Costa Carvalho, M.G., Wu, L., Califano, J., Westra, W., Jen, J. & Sidransky, D. (2001). *Cancer Res*, 61, 939-942.

Shi, H., Wei, S.H., Leu, Y.W., Rahmatpanah, F., Liu, J.C., Yan, P.S., Nephew, K.P. & Huang, T.H. (2003). *Cancer Res*, 63, 2164-2171.

Sidransky, D. (2002). *Nat Rev Cancer*, 2, 210-219.



Sirchia, S.M., Ren, M., Pili, R., Sironi, E., Somenzi, G., Ghidoni, R., Toma, S., Nicolo, G. & Sacchi, N. (2002). *Cancer Res*, 62, 2455-2461.

Smiraglia, D.J., Rush, L.J., Fruhwald, M.C., Dai, Z., Held, W.A., Costello, J.F., Lang, J.C., Eng, C., Li, B., Wright, F.A., Caligiuri, M.A. & Plass, C. (2001). *Hum Mol Genet*, 10, 1413-1419.

Spanjaard, R.A., Ikeda, M., Lee, P.J., Charpentier, B., Chin, W.W. & Eberlein, T.J. (1997). *J Biol Chem*, 272, 18990-18999.

Spugnardi, M., Tommasi, S., Dammann, R., Pfeifer, G.P. & Hoon, D.S. (2003). *Cancer Res*, 63, 1639-1643.

Taback, B., Fujiwara, Y., Wang, H.J., Foshag, L.J., Morton, D.L. & Hoon, D.S. (2001). *Cancer Res*, 61, 5723-5726.

Takeuchi, H., Kuo, C., Morton, D.L., Wang, H.J. & Hoon, D.S. (2003). *Cancer Res*, 63, 441-448.

Toyooka, S., Maruyama, R., Toyooka, K.O., McLerran, D., Feng, Z., Fukuyama, Y., Virmani, A.K., Zochbauer-Muller, S., Tsukuda, K., Sugio, K., Shimizu, N., Shimizu, K., Lee, H., Chen, C.Y., Fong, K.M., Gilcrease, M., Roth, J.A., Minna, J.D. & Gazdar, A.F. (2003). *Int J Cancer*, 103, 153-160.

Ueki, T., Toyota, M., Sohn, T., Yeo, C.J., Issa, J.P., Hruban, R.H. & Goggins, M. (2000). *Cancer Res*, 60, 1835-1839.

Usadel, H., Brabender, J., Danenberg, K.D., Jeronimo, C., Harden, S., Engles, J., Danenberg, P.V., Yang, S. & Sidransky, D. (2002). *Cancer Res*, 62, 371-375.

Widschwendter, M., Berger, J., Hermann, M., Muller, H.M., Amberger, A., Zeschnigk, M., Widschwendter, A., Abendstein, B., Zeimet, A.G., Daxenbichler, G. & Marth, C. (2000). *J Natl Cancer Inst*, 92, 826-832.

Widschwendter, M. & Jones, P.A. (2002). *Oncogene*, 21, 5462-5482.

Appl. No. 10/809,965  
Amdt. Dated November 26, 2007  
Reply to Office Action of May 25, 2007

Attorney Docket No. 89212.0016  
Customer No.: 26021

Zochbauer-Muller, S., Fong, K.M., Virmani, A.K., Geradts, J., Gazdar, A.F. &  
Minna, J.D. (2001). *Cancer Res*, 61, 249-255.